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We have reported that an influenza virus-specific polypeptide produced in E.coli induced influenza virus-subtype-specific memory and secondary H-2 restricted CTL responses in mice. The cl3 protein is a hybrid protein of the first 81 amino acids of the NS1 viral non-structural protein and the HA2 subunit of the viral hemagglutinin. We now report results which show that target cells exposed to cl3 protein are lysed by virus-immmune CTL in a subtype-specific H-2 restricted manner. This suggests that this protein interacts with target cell membranes and is presented on the cell membrane in proper confirmation with H-2 antigens for recognization by the influenza virus-specific CTL. Further, we show that immunization with this molecule results in the induction of virus-specific CTL, which are protective, and this peptide induce CTL without the need for adjuvants.

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Summary

Virus specific cytotoxic T lymphocytes (CTL) recognize viral antigen in conjunction with products of the genes of the major histocompatibility complex (MHC); however, the nature of the viral antigen(s) recognized by specific CTL is not clear. Although identification of viral determinants recognized by CTL is an important issue, it has been difficult to demonstrate CTL recognition of target cells exposed to virion antigens. It has been demonstrated by our laboratory and others that virus-specific H-2 restricted CTL are required from recovery from influenza virus infection. Extensive studies have been conducted using influenza A virus infection as a model system, and two different subpopulations of CTL, cross-reactive and subtype-specific, are induced. We reported that an influenza virus-specific polypeptide (designated c13 protein) produced an E.coli induced H-1 subtype-specific CTL memory and secondary H-2 restricted CTL responses in mice. In this report we demonstrate the c13 protein can be added to target cells and render them susceptible to killing by subtype-specific CTL.

Results

Influenza virus-specific CTL lysis of target cells exposed to c13 . We examined the ability of c13 protein to render P815 cells susceptible to influenza virus-specific CTL. Effector cells were generated by stimulation of A/PR/8 virus-immune spleen cells with A/PR/8 virus-infected syngeneic spleen cells and by c13 protein. As shown in figure 1 in the accompanying manuscript #1. c13 protein treated P815 cells were lysed by A/PR/8 virus- and c13 protein-induced CTL. The level of lysis was dose dependent up to a concentration of 3 ug/ml. These results suggest that target cells treated with c13 displayed an H1 virus-subtype-specific determinant. We, therefore, prepared two different effector cells. PR8 (H1) virus-stimulated, PR8 virus-immune spleen cells, A/PC (H3) virus-stimulated A/PC virus-immune spleen cells. Both PR8-immune effectors and PC immune effectors effect or specifically lyse PR8 virus-infected target cells; (e.g. 63.8% and 66.2% respectively at E/T ratios of 10:1); however, c13 protein coated target cells showed little lysis by A/PC virus-induced A/PC virus-immune cells, (2.8% at E/T 10:1), whereas they were definitely susceptible to PR8 virus-stimulated PR8 virus-immune spleen cells (34.0% E/T 10:1). These findings suggest that c13 protein coated target cells were lysed by PR8 virus-specific CTL but not by either cross-reactive or A/PC virus-specific CTL.

Cold target inhibition study

We tried to inhibit the killing activity of effector cells by cold-target cells to further examine the antigen specificity of effector cells recognizing target cells coated with c13 protein. Lysis of c13 coated ^{51}Cr-labelled target cells was inhibited by both PR/8 (H1) virus and A/BZ (H1) virus-infected target cells as well as by c13 protein coated target cells. (see figure 2 in accompanying article #1). A/X31 (H3) virus-infected ^{51}Cr-labelled target cells were not lysed by PR8 virus-induced effector cells in the presence of old cold target cells infected with type A viruses, but were lysed in the presence of c13-coated are uninfecte cold-target cells. To determine the H-2 restriction of killing of c13 protein-coated target cells, effector cells were obtained from

BALB/c ($H\text{-}2^d$) C3H ($H\text{-}2^K$) mice immunized with PR8. After stimulation with virus-infected syngeneic spleen cells cytotoxicity was examined on virus-infected and cl3 protein-coated P815 ($H\text{-}2^d$) and BW5147 ($H\text{-}2^k$) cells. Table 1 in accompanying manuscript shows that lysis of virus-infected target cells was definitely restricted by the H-2 complex. The lysis of P815 cells coated with cl3 protein was manifested by effector cells from BALB/c mice by not by those of C3H mice. To further examine a possible association of cl3 with the $H\text{-}2^d$ molecule, we attempted to block the killing of cl3 protein coated P815 cells by $H\text{-}2^d$ using monoclonal antibody directed to $H\text{-}2K^d$ and D^d. As shown in table 2 of the accompanying paper, the lysis of P815 cells infected with PR8 15 virus $H\text{-}2^d$ reduced in the presence of anti-$H\text{-}2^d$ monoclonal antibody. The $H\text{-}2^d$ monoclonal antibody inhibited BALB/c effector mediated lysis of cl3 protein coated P815 cells but did not effect the killing of PR/8 virus-infected BW5147 cells by C3H effectors. These results strongly suggest that cl3 protein is recognized by influenza virus-specific CTL in conjunction with $H\text{-}2K^d$ or D^d molecules. BW5147 cells coated with cl3 protein were not senstive to the lytic activity of C3H derived effectors cells even though they could lyse PR8 virus-infected BW5147 cells.

In our initial experiments we presumed that this protein would require potent adjuvants for the induction of immune responses, and we demonstrated induction of subtype-specific CTL following administration of 50 mcg of protein emulsified in an equal volume of complete Freund's adjuvant followed by intraperiteneal boosting three weeks later with 50 mcg of protein without adjuvant. Those results indicated that a subtype-specific CTL response was induced in the recipients of cl3 protein, and those data are contained in table 3 of the second accompanying manuscript. Subsequently we performed experiments using the Threonine derivitive of MDP kindly supplied by Dr. Anthony Allison, and also studied the effect of immunization with the 50 ug of protein without any adjuvants. Initially the MDP derivitive was compared to complete Freund's adjuvant in mice immunized with the cl3 protein; mice given cl3 and either of these adjuvants developed similar CTL responses. Subsequently, we have immunized animals with cl3 protein alone and found comparative levels of CTL memory induced indicating that the molecule itself can be presented appropriately and a CTL effector response can be demonstrated without adjuvants.

Ongoing and future research will concentrate on the biological role of these subtype-specific cross-reactive CTL in recovery and prevention of virus infections.